Journal of Photochemistry & Photobiology, B: Biology 238 (2023) 112619

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Cardiopulmonary and hematological effects of infrared LED photobiomodulation in the treatment of SARS-COV2

Pâmela Camila Pereira [a,b], Carlos José de Lima [a,c], Adriana Barrinha Fernandes [a,c], Renato Amaro Zângaro [a,c], Antonio Balbin Villaverde [a,c,*]

[a] Anhembi Morumbi University (UAM), Institute of Biomedical Engineering, Estrada Dr. Altino Bondensan 500, Distrito de Eugênio de Melo, CEP: 12.247-016 São José dos Campos, SP, Brazil

[b] University Center of Itajubá – (FEPI), Rua Dr. Antônio Braga Filho 687, Bairro Varginha, CEP: 37501-002 Itajubá, MG, Brazil

[c] Center of Innovation, Technology and Education – (CITE), Estrada Dr. Altino Bondensan 500, Distrito de Eugênio de Melo, CEP: 12.247-016 São José dos Campos, SP, Brazil

ARTICLE INFO

Keywords:
SARS-COV2
Photobiomodulation
Near-infrared LED
Cardiopulmonary functions
Hemogram

ABSTRACT

Background: COVID-19 disease is caused by SARS-CoV-2 which can trigger acute respiratory syndrome, which presents with dense alveolar and interstitial infiltrates and pulmonary edema, causing severe hypoxemia and significant alteration to pulmonary mechanics with reduced pulmonary compliance. The photobiomodulation technique alters cellular and molecular metabolism, showing promising results regarding the reduction of acute pulmonary inflammation.

Objective: To compare the photomodulation technique using near-infrared LED to conventional respiratory physiotherapy treatment in patients with COVID-19 in reversing acute conditions, reducing hospitalization time, and decreasing the need for oxygen therapy.

Methodology: The cohort was comprised of 30 patients undergoing COVID-19 treatment who were divided and allocated into two equal groups randomly: the LED group (LED), treated with infrared LED at 940 nm and conventional therapy, and the control group (CON), who received conventional treatment (antibiotic therapy for preventing superimposed bacterial infections, and physiotherapy) with LED irradiation off. Phototherapy used a vest with an array of 300 LEDs (940 nm) mounted on a 36 cm × 58 cm area and positioned in the patient's anterior thoracic and abdominal regions. The total power was 6 W, with 15 min irradiation time. Cardiopulmonary functions and blood count were monitored before and after treatment. The patients were treated daily for 7 days. Statistical analysis was conducted using a two-tailed unpaired Student's *t*-test at a significance level of $\alpha = 0.05$.

Results: Post-treatment, the LED group showed a reduction in hospital discharge time and a statistically significant improvement for the following cardiopulmonary functions: Partial Oxygen Saturation, Tidal Volume, Maximum Inspiratory, and Expiratory Pressures, Respiratory Frequency, Heart Rate, and Systolic Blood Pressure ($p < 0.05$). Regarding blood count, it was observed that post-treatment, the LED group presented with significant differences in the count of leukocytes, neutrophils, and lymphocytes.

Conclusion: Photobiomodulation therapy can be used as a complement to conventional treatment of COVID-19, promoting the improvement of cardiopulmonary functions, and minimization of respiratory symptoms.

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which promotes dyspnea, pulmonary edema, and pneumonia. Morbidity and mortality are associated with acute respiratory distress syndrome (ARDS) and cytokine storm. Patients hospitalized with COVID-19 are classified as severe if they require admission to the intensive care unit (ICU) [1,2].

The structure of the SARS-CoV-2 virus is represented by the envelope protein, protein E, hemagglutinin-esterase, protein M, protein S (spike),

* Corresponding author at: Center of Innovation, Technology and Education – CITE, Estrada Dr. Altino Bondensan 500, Distrito de Eugênio de Melo, CEP: 12.247-016 São José dos Campos, SP, Brazil.

E-mail address: abvillaverde@gmail.com (A.B. Villaverde).

https://doi.org/10.1016/j.jphotobiol.2022.112619
Received 13 March 2022; Received in revised form 28 November 2022; Accepted 2 December 2022
Available online 5 December 2022

and protein N. The functions performed by the S and N proteins are crucial in the pathogenesis of COVID-19. The S protein is anchored to ACE2 receptors (angiotensin-converter enzyme 2) for subsequent entry into the respiratory epithelial cell pneumocytes. The N protein, in addition to being responsible for viral replication, is largely produced during infection, and constitutes the main cause of the virus's high immunogenicity [3].

Infected patients present different symptoms lasting on average between 5 and 8 days, depending on the severity of the disease [4]. The mean time between the onset of symptoms and hospitalization ranges from 2 to 8 days, and the need for invasive mechanical ventilation (IMV) was 11 days, with 23.7 days on average until death (88% of cases) [5].

Acute respiratory syndrome is characterized by diffuse alveolar damage and by the development of noncardiogenic pulmonary edema, due to the increased permeability of the pulmonary alveolo-capillary membrane. Its clinical expression is hypoxemic respiratory failure and bilateral pulmonary infiltrate. Dependent pulmonary areas present dense alveolar and interstitial inflammatory infiltrate, edema, cellular debris, atelectasis, and consolidation, while non-dependent areas are relatively unaffected. It causes severe hypoxemia and accented alteration of pulmonary mechanics with a significant reduction of pulmonary compliance [6]. Recent studies of the action mechanism of the virus have shown that it causes a systemic infection that significantly affects the hematopoietic system and hemostasis [7,8].

In Brazil, medication protocols are following other health institutions across the world, using hydroxychloroquine [9] with a combination of antibiotics, such asteicoplanin [10] or azithromycin [11], used for preventing secondary infections that are present in many cases (such as sepsis). Nevertheless, the side effects of such medication are still under discussion in the literature. Patients who progress to dyspnea and respiratory discomfort require hospitalization and oxygen therapy according to advice from the World Health Organization (WHO) [12].

The use of light radiation in the red/infrared region is a noninvasive therapeutic intervention for the treatment of numerous lung diseases. Several studies in animal models, as well as in humans, demonstrate the effects of photobiomodulation using low-intensity lasers and LEDs in wound therapy, reducing the infectious and inflammatory processes, decreasing edema and inflammatory cells, stimulating microcirculation, and encouraging the formation of new vessels [13,14].

The photobiomodulation technique can modify cellular and molecular metabolism, signaling, reducing inflammation and release of chemical messengers, with promising results in reducing acute pulmonary inflammation, as they present significant potential for local balancing of immune responses [15,16]. In the last few decades, photobiomodulation has been used in the treatment of community-acquired pneumonia (CAP) with promising results regarding pulmonary inflammatory response and significant effects on the recovery of patients' blood count [17].

At the appropriate dose and wavelength, light interacting with cells and tissues can induce cellular functions such as lymphocyte stimulation, mast cell activation, an increase in mitochondrial ATP production and the proliferation of various cell types, thus promoting anti-inflammatory effects, such as the cytokines Interleukin 10 (IL-10), Interferon-gamma (INF-g), interleukin 1 (IL-1) and tumor necrosis factor (TNF-a) [18,19]. Brito et al. stated that photobiomodulation may even have an antifibrotic effect by decreasing TGFβ (transforming growth factor beta) in fibroblast cells and lung tissue [20].

The reduction in inspiratory muscle strength (PIM) is observed because of transient changes in the mechanical properties of the chest wall and respiratory muscles after critical illness and it is attributed to post-intensive care syndrome, which is characterized by the presence of physical, cognitive impairment or mental illness in patients undergoing prolonged ICU stay, including those with COVID-19. Another possible explanation for respiratory weakness could be the occurrence of interstitial lung disease after COVID-19 [21].

Recently, several studies have analyzed the possibility of employing the technique of photobiomodulation in the treatment of COVID-19 based on its potential to induce local and systemic effects, significantly decreasing pro-inflammatory cytokines, inflammatory cells, and collagen fiber deposition in the pulmonary parenchyma, enabling the reduction of mortality in patients [22–27].

The current scientific literature available contains few experimental studies on the effects of photobiomodulation on COVID-19. It is important to mention some of the pioneering articles that reported on the use of photobiomodulation in the treatment of COVID-19, which are: two case studies of patients with COVID-19 treated with 808 and 905 nm pulsed laser beams reported by Sigman et al., one patient 57 years old [28] and the other of 32 years old [29], and a preliminary study of 10 patients irradiated by pulsed lasers of 808 and 905 nm plus conventional medical treatment [30]. A clinical study with a larger cohort of 30 patients who were treated with pulsed lasers of 808 and 663 nm combined with a static magnetic field was investigated by de Marchi et al. [31].

In this context, aiming to improve the clinical recovery of patients, the present study proposes verifying the performance of the photobiomodulation technique with low-intensity light, using near-infrared LED, when compared to the conventional treatment of respiratory physiotherapy in patients with COVID-19. The treatment target is the reversal of the acute clinical condition of the COVID-19 patient by minimizing symptoms, reducing the need for oxygen therapy, and decreasing hospitalization time.

2. Methods

2.1. Ethical Concerns

This study was performed in line with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Anhembi Morumbi University (CAAE; 36,988,320.5.0000.5492) and registered with the Brazilian Registry of Clinical Trials (ReBEC) under the code U1111–1261-1981 (16/11/2020). Patients signed a free and informed consent form before the beginning of treatment.

This is a prospective, descriptive, single-blinded, randomized, and longitudinal trial conducted in the Respiratory Syndrome Ward of Santa Casa de Itajubá-MG.

2.2. Cohort

The cohort consisted of 30 patients admitted to the ward undergoing treatment for COVID-19. They were selected according to the following inclusion criteria: both sexes, aged between 50 and 80 years, with a clinical diagnosis of COVID-19, and in respiratory physiotherapy and medication. Exclusion criteria: patients who were from long-term institutions, overweight (Body Mass Index - BMI: above 29.9 kg/m^2), aged out of the study range, presented with neoplasms, or who have a history of photosensitivity.

An evaluation was performed shortly after each patient's hospitalization, which consisted of the acquisition of personal, sociodemographic, and clinical data (respiratory rate, heart rate, pulse oximetry, body temperature, diastolic and systolic pressures, and auscultation). The patient's clinical conditions were assessed daily for the seven days of treatment and the information was registered on a patient's record card. Positive reverse polymerase chain reaction (RT-PCR) tests, hospitalization stay time (in days), blood count, ventilometry (tidal volume), and respiratory muscle strength (manovacumetry) were evaluated before and after the seven days of intervention. Treatment and clinical condition evaluation were initiated soon after hospitalization.

Clinical criteria evaluated by a specialist physical therapist were adopted to determine the minimization of symptoms. The patients included in the present study were not intubated, breathing spontaneously, or using oxygen therapy up to 3 L/minO_2. The symptoms evaluated were cough (presence or absence), fever, risk of dyspnea on minimal exertion or at rest, respiratory rate > 22 breaths/min, and SpO_2

of <90% with supplemental oxygen.

2.3. Pneumonia Severity Index – PSI and Neutrophil/Lymphocyte Count Ratio - NLCR

Pneumonia severity index score is a significant scoring system for predicting the disease severity of patients with pulmonary infections, to indicate the patient's hospital admission status, and mortality risk. The PSI scoring system covers 20 variables that include demographic characteristics, associated diseases, laboratory and radiological alterations, and physical examination findings. The total score of the variables allows the stratification of severity into five classes, based on the risk of death and the need for hospitalization [32].

The neutrophil-to-lymphocyte count ratio (NLCR) is another valuable parameter used in clinical practice to evaluate the severity of pulmonary infections, such as CAP [33] and COVID-19 [34], at a patient's hospitalization. According to Hassan et al. [34], the NLCR can be recommended as a highly sensitive and specific indicator for severity prediction in Covid-19 patients. In the present study, it was used as a predictor of disease severity and treatment outcome. The higher the NLCR value, the higher the concentration of inflammatory cytokines (IL-2, IL-6 and IL-10) and the higher the IgG values and, consequently, greater severity with a worse prognosis [35]. As usual, neutrophil count increases, and lymphocyte count decreases with the advancement of any inflammatory condition. Values of NLCR below 10 indicate a low to moderate degree of disease severity [33].

2.4. Complete Blood Count

A complete blood count test (CBC) was performed, including an assessment of erythrocytes counts, hemoglobin and hematocrit concentrations, leukocytes, and platelet counts. The test was performed by the Syrius Medical Group Laboratory of Clinical Analyses using an automated XS-800i model (Sysmed, Curitiba, Brazil). Pneumonia Severity Index (PSI) and the neutrophil-to-lymphocyte count ratio (NLCR) were assessed at the time of hospital admission to determine the sickness severity risk of the patients. NLCR values were again assessed after treatment to verify the response (positive or negative) to therapy. The CBC test was conducted at the time of patient hospitalization and repeated the day after the last LED irradiation.

2.5. Ventilometry

The ventilometry evaluation was performed by means of a ventilometer Mark Wright 8 ® (AAMED – Comércio de Equipamentos - Campo Belo - São Paulo, Brazil) with the placement of oral and nasal clips. The patient was requested to inhale and exhale relaxedly, and the volume of air that entered and left the lungs at each respiratory cycle was assessed (mL).

2.5.1. Maximum Inspiratory Pressure

The maximum inspiratory pressure (MIP) was measured by the manovacuometer device Spire® (Murena's Produtos para a Saúde Ltda-Me- Progresso, Juiz de Fora-MG, Brazil). During the test, the patients remained seated with their nostrils occluded by a nasal clip, and the individuals firmly held the mouthpiece against their lips, avoiding air leakage. Patients were instructed to perform a maximum expiration and then a verbal command was given for the patient to perform a maximal inspiratory effort sustained for at least 2 s. MIP was measured, in cmH_2O, during the exertion initiated from the residual volume (RV). At least three satisfactory measurements of each pressure were taken; that is, without air leakage through the mouth or nose and with values close to each other, being used only the highest value.

2.5.2. Maximum Expiratory Pressure

The maximum expiratory pressure (MEP) was also measured using the manovacuometer Spire® (Murena's Produtos para a Saúde Ltda-Me-Progresso, Juiz de Fora-MG, Brazil). The MEP was obtained from the total lung capacity (TLC), in which the patient is requested to engage maximum inspiration before maximum expiratory effort, with minimum support of 2 s, MEP was measured in cmH_2O. At least three satisfactory measurements of each pressure were performed following the same protocol as for MIP, and only the highest value was used.

2.6. Vital Signs Monitoring

2.6.1. Respiratory Rate

The normal value in adults is 12 to 20 inspirations per minute. The test was performed by means of a ventilometer Mark Wright 8 ® (AAMED – Comércio de Equipamentos - Campo Belo - São Paulo, Brazil); oral and nasal clips were placed on the patient, who was requested to inhale and exhale relaxedly for 1 min.

2.6.2. Heart Rate

Heart rate (HR) is the number of times that the heart beats per minute. At rest, the normal values for HR range from 60 to 100 bpm. An oximeter Model SB1000® (Medical Rossmax, Taiwan) was used to assess heart rate.

2.6.3. Blood Pressure

Blood pressure (BP) is the force with which the heart pumps blood through the vessels. It is determined by the result of the product cardiac output x peripheral vascular resistance. There are two pressures: the maximal, or systolic, which is when the heart contracts, and the minimum, or diastolic, which is when the heart dilates. Blood pressure was assessed by a calibrated analog manometer of BIC® brand (Manaus, Brazil), certificated by the Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO) of Brazil, and a Littman Lightweight stethoscope™ (Model 2454, 3 M manufacturers, Nova Vezena – SP, Brazil). Normal values for blood pressure are systolic <120 mmHg and diastolic >90 mmHg.

2.6.4. Pulse Oximetry

The oxygen level measured with an oximeter is called the oxygen saturation level (SaO_2), which is the percentage of oxygen that the blood is carrying, compared to the maximum carrying capacity. Oxyhemoglobin saturation is measured by using the Rossmax® pulse oximetry (SpO_2), with a normal value between 90 and 100%.

2.6.5. Body Temperature

Body temperature was evaluated for one minute by means of the g-tech digital infrared thermometer G-Tech® (model FR1DZ1, Accumed Produtos Medico-Hospitalares Ltda, Duque de Caxias-RJ, Brazil). The three thermal states include euthermia, hypothermia, and hyperthermia.

2.6.6. Pulmonary Auscultation

Evaluation of pulmonary sounds was conducted via the Littman Lightweight stethoscope™ (Model 2454, 3 M manufacturers, Nova Vezena – SP, Brazil), checking whether sounds are altered in frequency and intensity. It was performed in the anterior region of the thorax symmetrically in the following foci: two fingers below the clavicle, medially to the sternum bone, and laterally to the last ribs.

2.7. Treatment Protocol

The cohort of 30 patients with COVID-19 was randomly divided into two equal groups. The LED group comprised patients treated with conventional therapy (medication and physiotherapy) in conjunction with infrared LED irradiation (940 nm), and the CON (placebo) group comprised patients that underwent conventional therapy alone.

Patients from both groups received conventional treatment consisting of the antibiotics Meropenem (1-2 g) or Tazocin (40 mL/min), in conjunction with respiratory physiotherapy for bronchial hygiene with a 15 Hz oral oscillation device (OOAF, Shaker, NCS, São Paulo, Brazil). Respiratory physiotherapy of 30 min, consisting of three cycles of 10 repetitions each, with an interval of 1 min between cycles, was performed daily before wearing the LED vest, during the 7 days of treatment with LED irradiation or placebo. After that time, the two groups continued only with conventional treatment until discharged from the hospital.

The control group undertook the same protocol as the LED group, but they used the infrared LED vest with the LEDs turned off. Blinding was obtained because infrared radiation is not perceived by the human eye and patients from both groups wore the vest for the same time (15 min).

Fig. 1 depicts a flow diagram of the stages of the experimental protocol from cohort selection, distribution between the two groups, treatment description, and evaluation tests to determine the treatment progress.

The LED irradiation protocol is described in Pereira and coworkers' article [17]. Briefly, the LED system consisted of a set of 300 infrared LEDs (940 nm) with an optical power of 0.02 W each. The LEDs were arranged in a network-like distribution, with LEDs spaced at 2 cm (horizontal) × 4 cm (vertical) and positioned in the anterior thoracic and abdominal regions of the body via a vest with a total area of 2088 cm^2, that was coated with a transparent plastic film to allow cleaning and sanitization. The patients were irradiated for 15 min, one session per day, for 7 consecutive days. The LED system parameters over the vest area were total optical power of 6 W and an average power density of 2.9 mW/cm^2, corresponding to 5.4 kJ total optical energy during the 900 s of irradiation time. The vest with LEDs and the patient wearing the vest are shown in Fig. 2.

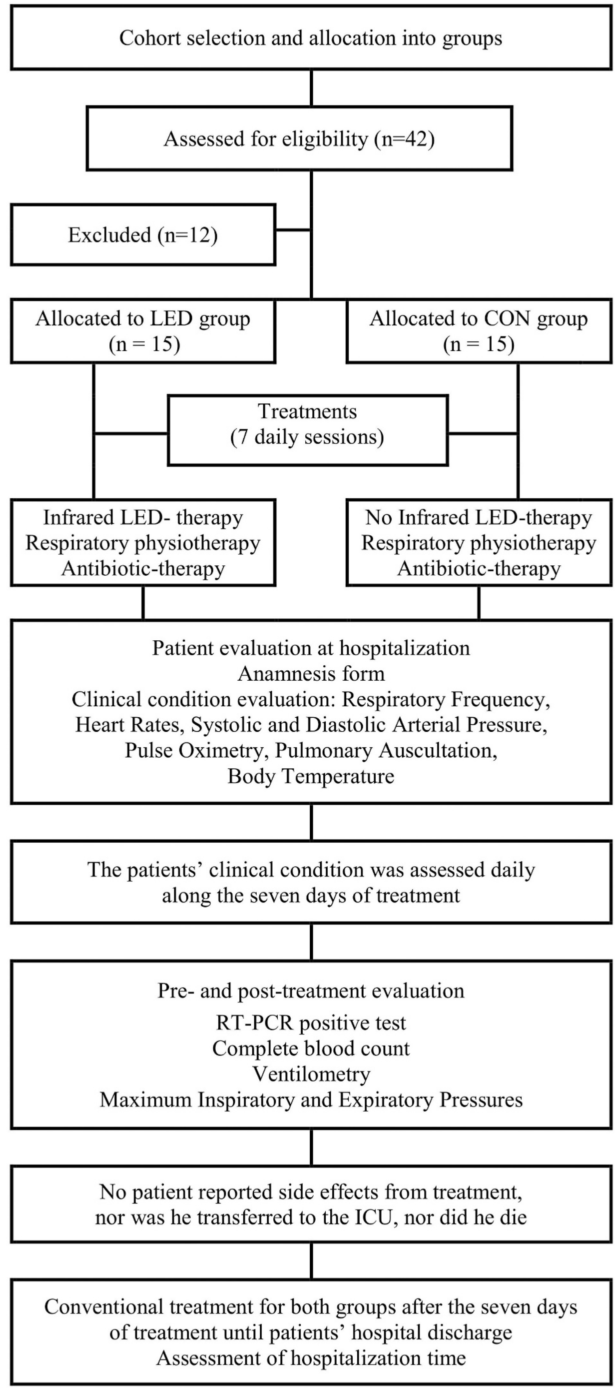

Fig. 1. Flow diagram showing the cohort distribution and the sequence of the stages of the experimental protocol.

2.8. Evaluation of the Treatment Progress

To compare the efficacies of the LED and CON therapies, two new differential variables were defined:

ΔLED (variable) = parameter value in the post-treatment - value in the pre-treatment, for the LED group.

Similarly, the ΔCON was defined as ΔCON (variable) = parameter value in the post-treatment - value in the pre-treatment, for the CON group.

These differential variables were used to overcome statistical bias due to large data dispersion among patients in the baseline group.

2.9. Statistical Analysis

The normality of the data was verified by the Kolmogorov–Smirnov normality test for both groups. A parametric two-tailed paired *t*-test was used for the intra-group analysis of the data before and after treatment of each group; whereas the inter-group statistical analysis of the differential variables ΔLED and ΔCON was conducted via the parametric two-tailed unpaired *t*-test, followed by a Welch correction when applicable. Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for the intra-group and inter-group analyses, with a significance level of $\alpha = 0.05$. The number of men and women in each group and the morbidities distribution in each group were compared using Fisher's exact test and the Chi-squared test for trends (both at $\alpha = 0.05$), respectively. Data are expressed as the mean ± SEM.

The Cohen's d effect size parameter was used to calculate the statistical power of the two-tailed *t*-test of ΔLED vs. ΔCON, when a statistically significant difference was achieved. According to Sawilowsky's classification of effect size, d(0.8) = large, d(1.2) = very large, and d (2.0) = huge [36].

3. Results

A total of 30 patients participated in the study, equally allocated into LED and CON groups. Table 1 shows the values of the patients' hospital intake (mean and SEM) of BMI and age for each group, as well as the number of men and women in each group. A comparison of the distribution of the number of men and women between groups showed that both groups were homogeneous ($p = 1.000$). Similarly, no significant differences were found between the two groups regarding BMI ($p = 0.70$) and age ($p = 0.10$). Patients from both groups presented with different associated diseases: high blood pressure (HBP), chronic kidney disease (CKD), diabetes mellitus (DM), and heart failure (HF). Statistical analysis shows that patients' morbidities distribution between both groups was homogeneous ($p = 0.542$).

Pneumonia severity index (PSI) values were calculated for all

Fig. 2. Photographs showing the LED-therapy protocol (Left) view of the open vest showing the array of LEDs emitting infrared radiation. The vest was mounting with 300 infrared LEDs (GaAlAs, 940 nm) of 5 mm diameter, emitting with a divergence angle of 30°, and an optical power of 0.02 W each (Model TSAL6400, Vishay Semiconductors, Vishay Intertechnology Ltd., Singapore). The LEDs were arranged spaced at 2 cm (horizontal) × 4 cm (vertical), with 10 lines and 30 columns. The entire system was constructed in our own laboratory. The LED system parameters over the vest area were total optical power 6 W, average power density 2.9 mW/cm^2, SAEF (Surface average fluence) 2.6 J/cm^2, and 5.4 kJ total optical energy during the 900 s of irradiation time. (Right) view of the patient wearing the vest positioned in the anterior thoracic and abdominal regions of the body, into direct contact with the skin, during the infrared irradiation process. The vest size was 36 cm × 58 cm, covering an area of 2088 cm^2.

Table 1
Data at the patients' hospital intake: body mass index (BMI), age, pneumonia severity index (PSI), sex, and comorbidities.

Groups	Mean		SEM		p
Body mass index - BMI (kg/m^2)					
LED	26.1		2.0		0.704$^+$
CON	25.6		0.5		
Age (years)					
LED	66.9		2.3		0.096^{++}
CON	62.3		2.1		
Pneumonia severity index (PSI)					
LED	97.1 (IV)		1.5		0.003$^+$
CON	85.3 (III)		3.2		
Sex (M/W)	Men		Women		p
LED	8		7		1.000^{+++}
CON	7		8		
Comorbidities (number of patients)	HBP	CKD	DM	HF	p^{IV}
LED	12	1	5	0	0.542
CON	9	0	5	1	

p$^+$: parametric two-tailed unpaired t-test at α =0.05 (Welch correction).
p^{++}: parametric two-tailed unpaired *t*-test at α =0.05.
p^{+++}: two-tailed Fisher's exact test at α =0.05.
p^{IV}: Chi-Squared Test for Trend at α =0.05.
PSI classification: class III (71–90 points); class IV (91–130 points).
HBP: High Blood Pressure; CKD: Chronic Kidney Disease; DM: Diabetes Mellitus; HF: Heart Failure.

patients at the hospital intake. Mean values depicted in Table 1 indicated that the LED group presented with higher severity risk (class IV) than the CON group (class III). PSI is a scoring system that predicts the severity of the disease, but high PSI values do not prevent a better recovery of patients after treatment. In the present study, although the PSI of the LED group is higher than in the CON group, the LED group showed better recovery than the CON group, which reinforces the beneficial effect of the photobiomodulation.

Further, the hospital stay time for both groups, i.e., the time elapsed between patients' hospital intake and discharge, was measured (Mean ± SEM): LED (8 ± 0.2) days and CON (11.7 ± 1.4) days. That outcome reveals the effect of the photobiomodulation on reducing the time of hospital stay from 11.7 days to 8 days ($p = 0.02$).

Pulmonary auscultation, the time when adventitious noises (pulmonary snoring) improved from the date of patient hospital intake was measured for both groups, obtaining the results (Mean ± SEM): LED (3.5 ± 0.2) days, CON (5.6 ± 05) days, $p = 0.0006$, showing a statistically significant reduction on the time of pulmonary auscultation improvement in favor of the LED group.

The NLCR, index for the initial prognosis of the disease severity risk of patients with respiratory disorders, was calculated obtaining the results (Mean/SEM): group LED - 13.4 (2.8) and group CON – 16.2 (3.7), $p = 0.55^{ns}$. To corroborate the beneficial effect of the photobiomodulation with infrared LED, the NLCR values were re-calculated for both groups after treatment: LED 6.3 (1.5) and CON 11.9 (1.5), $p = 0.014$. The results obtained showed that the control group presented a reduction of 27% in NLCR values while the LED group reduced it by 53%. Thus, comparative analysis of NLCR values before and after treatment demonstrated the beneficial effect of the photobiomodulation with infrared LED, suggesting a systemic anti-inflammatory effect.

A set of different tests were carried out to conduct a thorough analysis of the progress of both treatments, either the conventional alone or in conjunction with the LED irradiation. These included the pulmonary functions: oxygen flow intake - O_2 (L/min), partial oxygen saturation - SpO_2 (%), Tidal volume - TV (mL), Maximum Inspiratory Pressure - MIP (cmH_2O), Maximal Expiratory Pressure - MEP (cmH_2O), Respiratory Frequency - RF (rpm); and the cardiological functions: Heart Rate – HR (bpm), Systolic Blood Pressure – SBP (mmHg), and Diastolic Blood Pressure – DBP (mmHg).

Regarding the performance of respiratory muscles, the control group showed significant improvement in MIP ($p = 0.0001$), but the same was not observed in the MEP ($p = 0.054$). On the contrary, the LED group presented a significant improvement in MIP (p = 0.0001) and MEP (p = 0.0001), indicating an enhancement in respiratory muscle performance and functional capacity in patients with COVID-19.

Hematological evaluation of the treatment was performed using the CBC test before and after treatment, assessing the following hematologic components: Erythrocytes (x10^6 mm^{-3}), Hemoglobin (mg/dL), Hematocrit (%), Leukocytes (mm^{-3}), Segmented Neutrophils (mm^{-3}), Lymphocytes (mm^{-3}), Monocytes (mm^{-3}), Eosinophils (mm^{-3}), and Platelets (x10^3 mm^{-3}). The tests were completed by the body temperature assessment of the patients. The function assessments were made at baseline (patients' hospital intake) and the day after the end of the infrared LED irradiation or placebo.

The data of each group passed the Kolmogorov–Smirnov normality test; hence, we used a paired two-tailed parametric *t*-test for the intragroup statistical analyses of both groups. Table 2 displays the data obtained from the tests applied to the two groups, before (baseline) and

Table 2
Cardiopulmonary, Hematologic, and Body Temperature data, Mean (SEM), with intra-group statistical analysis of groups.

	Group LED			Group CON		
	Baseline	Post-treatment	$p^{\dagger}$	Baseline	Post-treatment	$p^{\ddagger}$
Cardiopulmonary analysis						
Oxygen Flow Intake (L/min)	3.3 (0.3)	0.3 (0.1)	0.0001***	5.4 (0.6)	1.3 (0.3)	0.0001***
Partial Oxygen Saturation (%)	86.7 (0.3)	96.1 (0.3)	0.0001***	89.3 (0.6)	91.9 (0.5)	0.002**
Tidal Volume -TV (mL)	320 (16)	394 (15)	0.0001***	297 (8)	319 (9)	0.0001***
Maximum Inspiratory Pressure (cmH_2O)	−52.7 (2.3)	−77.1 (1.8)	0.0001***	−48.7 (1.8)	−55.7 (2.2)	0.0001***
Maximal Expiratory Pressure (cmH_2O)	63.5 (3.6)	82.7 (2.7)	0.0001***	62.3 (1.8)	64.3 (2.0)	0.054
Respiratory Frequency (rpm)	18.1 (0.5)	12.9 (0.2)	0.0001***	16.3 (0.2)	13.5 (0.2)	0.0001***
Heart Rate (bpm)	100.4 (4.4)	80.7 (2.2)	0.0001***	82.0 (1.8)	80.1 (1.8)	0.36
Systolic Blood Pressure (mmHg)	138 (2.7)	125 (2.4)	0.0001***	137 (2.2)	132 (1.3)	0.01*
Diastolic Blood Pressure (mmHg)	91.4 (1.3)	85.7 (1.2)	0.003**	87.1 (1.0)	83.9 (1.6)	0.1
Hematologic analysis						
Erythrocytes (x 10^6 mm^{-3})	4.10 (0.2)	4.21 (0.2)	0.64	4.65 (0.15)	4.55 (0.18)	0.3
Hemoglobin (mg/dL)	12.03 (0.5)	12.75 (0.5)	0.29	12.90 (0.47)	12.75 (0.39)	0.57
Hematocrit (%)	36.30 (1.1)	37.07 (1.5)	0.69	36.75 (2.1)	37.39 (1.1)	0.74
Leukocytes (mm^{-3})	10,943 (1320)	7412 (730)	0.004**	9086 (1100)	10,350 (1170)	0.091
Segmented Neutrophils (mm^{-3})	8300 (206)	7300 (253)	0.004**	8390 (197)	8270 (159)	0.5
Lymphocytes (mm^{-3})	950 (178)	1800 (234)	0.003***	890 (163)	890 (126)	0.96
Monocytes (mm^{-3})	390 (45)	460 (40)	0.21	347 (42)	327 (45)	0.38
Eosinophils (mm^{-3})	66.7 (20)	70 (15)	0.86	53.3 (16)	100 (33)	0.44
Platelets (x 10^3 mm^{-3})	219 (32)	301 (22)	0.67	188 (22)	199 (22)	0.14
Body Temperature						
Temperature (°C)	39.1 (0.1)	36 (0.1)	0.0001***	39.2 (0.1)	36 (0.1)	0.0001***

$p^{\dagger}$: intra-group statistical analysis for group LED; $p^{\ddagger}$ intra-group statistical analysis for group CON.
Parametric two-tailed paired *t*-test at the significance level of $\alpha = 0.05$. P-value: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
Reference values: Partial Oxygen Saturation (%) > 90, Maximum Inspiratory Pressure (cmH_2O) > −80, Maximal Expiratory Pressure (cmH_20) > 60, Respiratory Frequency (rpm) 1220, Heart Rate (bpm) 60-100, Systolic Blood Pressure (mmHg) 120–129, Diastolic Blood Pressure (mmHg) 80–89, Erythrocytes ($x10^6/mm^3$) 4.0–6.5, Hemoglobin (mg/dL) 12–16, Hematocrit (%) 36–45, Leukocytes (mm^{-3}) 4000–10,000, Segmented neutrophils (mm^{-3}) 2200–6600, Lymphocytes (mm^{-3}) 800–3500, Monocytes (mm^{-3}) 160–800, Eosinophils (mm^{-3}) 40–400, Platelets ($x10^3/mm^3$) 150–450, Body temperature (°C) 35–37.

after treatment. Table 2 includes the *p*-values given by the intra-group statistical analysis of post- versus pre-treatment for each group. The intra-group analysis showed that the LED group exhibited a statistically significant improvement after treatment for all the cardiopulmonary functions ($p < 0.05$); however, for the CON group, no significant differences were found for heart rate and diastolic blood pressure ($p > 0.05$). Regarding the analysis of blood count, it was observed that the LED group exhibited significant differences after treatment for the white blood cell count: leukocytes, neutrophils, and lymphocytes. On the other hand, the CON group showed no differences in blood count. It is worth mentioning that patients belonging to the LED and CON groups did not present, on average, with anemia, since their red cell series values were within the normal range at the time of hospitalization.

The inter-group statistical analysis of the differential variables ΔLED and ΔCON was conducted via the parametric two-tailed unpaired *t*-test, followed by a Welch correction when it was needed. Table 3 depicts the inter-group statistical analysis for ΔLED and ΔCON for all the studied tests, and the corresponding *p*-values of significance. It can be observed from Table 3 that treatment with LED irradiation significantly improved the effect of conventional treatment on the cardiopulmonary functions ($p < 0.0001$): SpO_2, TV, MIP, and MEP, ($p = 0.0009$) for RF, ($p = 0.0001$) for HR, and to a lesser degree the SBP function ($p < 0.007$). On the contrary, the patient O_2 intake was shown to be reduced more by the conventional treatment alone than when it was in conjunction with LED therapy, as seen in ΔLED versus ΔCON (Table 3). This can be explained by the fact that the initial need for O_2 was much greater for patients treated with conventional therapy, as can be seen in Table 2.

As for the effect of each treatment on the hemogram, there was a statistically significant difference between ΔLED versus ΔCON for Leukocytes ($p < 0.001$), and Segmented Neutrophils and Lymphocytes ($p < 0.01$). No difference between treatments was observed for the red cell series.

The power of the inter-group statistical analysis employing the *t*-test was calculated by using Cohen's d parameter. The values of this parameter are shown in Table 3 in cases where a statistically significant difference was found ($p < 0.05$). It can be observed from Table 3 that values of d range from 0.9 for oxygen flow intake and neutrophils up to 3.1 for partial oxygen pressure. It is worth mentioning that $d \geq 0.9$ values correspond to statistical powers >80%, i.e., type II errors $\beta < 20\%$ [37].

3.1. Outcomes Summary

Infrared LED photobiomodulation combined with conventional therapy outcomes:

- ➢ Enhances the effect of the conventional therapy on COVID-19 patients, presenting a statistically significant improvement in the recovery of the vital cardiopulmonary functions: Partial Oxygen Saturation, Tidal Volume, Maximum Inspiratory Pressure, Maximum Expiratory Pressure, Respiratory Rate, Heart Rate and Systolic Blood Pressure; as well as the hematological components: Leukocytes, Segmented Neutrophils and Lymphocytes.
- ➢ Statistically significant reduction in the time of hospitalization stays of patients.
- ➢ The time when adventitious noises improved from the date of the patient's hospital intake is significantly reduced
- ➢ Presents an improvement in the PSI and NLCR indices when compared to conventional therapy.
- ➢ The power of statistical analysis of the results exceeded 80%

4. Discussion

This clinical trial demonstrated the beneficial effects of photobiomodulation in patients with COVID-19 symptoms, corroborating other data reported in the scientific literature on the anti-inflammatory

Table 3
Cardiopulmonary, hematologic, and body temperature outcomes, Mean (SEM), inter-group statistical analysis between groups, and Cohen's parameter.

	ΔLED	ΔCON	p	d
Cardiopulmonary analysis				
Oxygen flow intake (L/min)	−3.1 (0.2)	−4.1 (0.4)	0.025*	0.9
Partial Oxygen Saturation (%)	9.4 (0.5)	2.6 (0.7)	< 0 0.0001***	3.1
Tidal Volume (mL)	74.4 (9.0)	22.1 (3.3)	< 0.0001***	2.0
Maximum Inspiratory Pressure (cmH_2O)	−24.9 (2.2)	−7.0 (0.8)	< 0.0001***	2.8
Maximal Expiratory Pressure (cmH_2O)	19.1 (3.0)	2.0 (0.9)	< 0.0001***	2.0
Respiratory Frequency (rpm)	−5.1 (0.5)	−2.8 (0.4)	0.0009***	1.4
Heart Rate (bpm)	−19.7 (3.3)	−1.9 (1.5)	0.0001***	1.7
Systolic Blood Pressure (mmHg)	−13.6 (2.3)	−5.3 (1.8)	0.007**	1.1
Diastolic Blood Pressure (mmHg)	−5.7 (1.6)	−3.1 (1.8)	0.30^{ns}	–
Hematologic analysis				
Erythrocytes (x 10^6 mm^{-3})	0.1 (0.2)	−0.1 (0.1)	0.41^{ns}	–
Hemoglobin (mg/dL)	0.7 (0.7)	−0.15 (0.3)	0.23^{ns}	–
Hematocrit (%)	0.8 (1.9)	0.6 (1.9)	0.96^{ns}	–
Leukocytes (mm^{-3})	−3531 (1030)	1270 (710)	0.0006***	1.4
Segmented Neutrophils (mm^{-3})	−930 (270)	−130 (190)	0.02*	0.9
Lymphocytes (mm^{-3})	850 (240)	−10 (130)	0.004**	1.2
Monocytes (mm^{-3})	70 (53)	−20 (23)	0.14^{ns}	–
Eosinophils (mm^{-3})	3.3 (18)	46.7 (39)	0.83^{ns}	–
Platelets (x 10^3 mm^{-3})	10.4 (24)	10.4 (7)	0.99^{ns}	–
Body Temperature				
Body Temperature (°C)	−3.2 (0.15)	−3.1 (0.15)	0.80^{ns}	–

ΔLED = (post-treatment – baseline) LED group; ΔCON = (post-treatment - baseline) CON group.
p: inter-group statistical analysis for group LED versus group CON.
parametric two-tailed unpaired *t*-test at the significance level of $\alpha = 0.05$.
ns: not significant $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
d: Cohen parameter; sample effect sizes d (0.8) = large, d (1.2) = very large, and d (2.0) = huge.
—— Cohen's parameter is not calculated when the difference is not statistically significant.

effects of this technique on lung tissues in both animal and human models [5,17,20,28–31,38,39].

In the present study, the PSI index was used to determine the initial prognosis of the severity of risk for patients with respiratory diseases. It was observed that patients in the LED group had a worse prognosis in relation to the CON group, LED 97.1 vs CON 85.3 ($p = 0.003$); it is noteworthy that the distribution of patients was randomly assigned. Further, the NLCR index showed a reduction of 53% for LED against 27% for CON, after the end of the treatment, indicating the beneficial effect of the photobiomodulation. The obtained data exhibited improvement in the clinical condition of the group after irradiation with infrared LED in oxygenation ($p = 0.0001$), inspiratory muscle strength ($p = 0.0001$), tidal volume ($p = 0.0001$), pulse oximetry ($p = 0.0001$), and respiratory rate ($p = 0.0001$), The total clinical recovery of patients in the CON group on average took 11.7 days of hospitalization, while patients treated with LED took only 8 days ($p = 0.021$); the mean time for COVID-19 is usually 6–8 weeks [40]. In a study by Vetrici et al., the average number of days hospitalized in the photomodulation group was 7.6 days compared to 12.2 days for the control group ($p = 0.292$) [30]; these data corroborate those obtained in the present study.

Improvement in respiratory function was demonstrated in patients treated with LED in relation to the control group, as evidenced by the tests assessing ventilometry, manovacumetry, and peripheral oximetry. In a study by Sigman et al., partial oxygen saturation increased significantly from 94% to 100% in the first 5 min of irradiation and then remained at the recommended levels after that period [28]. Vetrici et al. demonstrated that patients in the control group and PBM group presented with fluctuations in their pulmonary function; however, PBM patients did not require ICU admission or mechanical ventilation. In addition, all PBM patients no longer required O_2 support 9 days after initiation of treatment [30].

When comparing the values obtained for MIP and MEP of both groups (Table 3), it was verified that the LED group enhanced the performance of the respiratory muscles compared to the control group ($p = 0.0001$). These data suggest that photomodulation preserved the main respiratory muscle, which facilitated the ventilation/perfusion process, promoting an improvement of the clinical and ventilatory status of patients. These important findings indicate that data obtained in the present study corroborate de Marchi et al. [31] results, because a significant improvement in inspiratory muscle strength (MIP) was observed.

In the study by Tomazoni et al., photomodulation therapy alone or combined with a static magnetic field (PBMT-sF) was performed with irradiation in the lower chest, upper abdominal cavity, and two sites in the neck of the patients [41]. It was observed that the patients were able to leave oxygen support during treatment, increasing peripheral oxygen saturation, and showed an improvement in pulmonary severity scores and radiological findings [41]. These data agree with those obtained in the present study, showing that photobiomodulation was effective in improving the pulmonary functional capacity of the patients.

Photobiomodulation is useful for cellular metabolism and to proliferate or improve lung tissue, according to a report by Nejatifarda et al. [26]. They observed significant decreases in pulmonary edema, the neutrophil influx, and the generation of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), intracellular reaction molecule (ICAM), reactive oxygen species (ROS), nitric oxide synthase isoform (iNOS) and macrophage inflammatory protein 2 (MIP-2). These findings demonstrate that photomodulation can be useful in reducing pulmonary inflammation and promoting the regeneration of damaged tissue as well as minimizing the sequelae of pulmonary fibrosis caused by COVID-19 [31].

A statistically significant reduction was observed in this study for the count of leukocytes, neutrophils, and lymphocytes after therapy when infrared LED was combined with conventional treatment ($p < 0.05$); however, no significant reduction was observed in patients who received only medication and physiotherapy ($p > 0.05$). In a study by Pereira et al. [17], patients with community-acquired pneumonia (CAP) who were treated with LED therapy showed a significant reduction in the number of leukocytes, neutrophils, lymphocytes, and monocytes.

The eosinophils are leukocytes tissue-resident and circulating that have potent proinflammatory effects, and antiviral and immune regulation activity. Nair et al. [42] reported in their study that the eosinophil count showed to be very variable in patients with COVID-19, with a relatively high prevalence of eosinophilia in symptomatic COVID-19 positive patients. According to Anka et al. [43], patients with severe COVID-19 present eosinopenia and lymphopenia. However, in the present study, the eosinophil counts were within the reference values (40 to 400 mm^{-3}) for both groups.

Data from this study and those reported by Pereira et al. [17] agree with the findings of de Brito et al. [20], who verified a systemic effect of LLLT (780 nm and 30 mW) on the reduction of inflammatory cell counts in the blood of animals with idiopathic pulmonary fibrosis, noting that, in relation to cytokines, LLLT reduced the release of pro-inflammatory cytokines and increased IL-10, justifying the anti-inflammatory effect presented by the photobiomodulation [20].

Several studies have recommended the use of corticosteroids for the treatment of COVID-19, and they have been included in therapeutic protocols due to their anti-inflammatory, antifibrotic, and vasoconstrictive effects, which reduce the systemic effects of the disease [44,45]. The WHO React Working group [46] reported that corticosteroids reduced mortality and ventilatory support time; dexamethasone reduced the number of deaths by approximately 36%, and hydrocortisone by 31%. The action of methylprednisolone was slightly lower, reducing mortality by 9%. The present study showed that the LED group had reduced oxygen therapy time, decreased risk of complications, and less lung damage, suggesting a promising clinical use for photomodulation using infrared LED irradiation in intensive care units and wards. This could allow the reduction or non-use of corticosteroids, since these drugs have adverse effects such as toxicity, in addition to requiring vital organs such as the liver and kidneys to metabolize or excrete drugs, which is not the case with phototherapy.

No reported side effects or complications associated with LED therapy were observed during treatment, and no patients died. Due to the severity of the disease of patients with COVID-19, the use of LED therapy can improve clinical status and reduce the need for ICU beds and oxygen intake and, consequently, the use of mechanical ventilators. Other potential benefits of LED therapy include that the treatment is an easy, safe, non-invasive, non-pharmacological, painless, and low-cost modality. The results of this study are promising and will stimulate further research to evaluate the direct effect of photobiomodulation on the pulmonary condition of patients with COVID-19. It is worth mentioning that, in the present study, the pulmonary function of the groups was evaluated by objective measures, which is relevant because this approach has not been supported so far in the current literature.

Among the strengths of this study, it should be noted that it is innovative because employed phototherapy using a vest with an array of 300 LEDs (940 nm) in complement to the conventional treatment of COVID-19. Moreover, the photobiomodulation reduced the average hospitalization time by four days and induced a significant improvement in the MIP (32%) and MEP (23%) pulmonary functions, these data are very promising and highlight the systemic effect of photobiomodulation.

The main limitation of the present study was the size of the cohort of patients, a larger participation of patients in the study should increase the strength of the statistical analysis. Other possible items that could be investigated, such as filling out questionnaires by patients, monitoring the process of pulmonary inflammation, testing different doses of LED irradiation and wavelengths, and others, were left for future research because the study was developed during the peak of the COVID-19 pandemic when the whole effort was to find innovative therapies for the better recovery of the patients.

5. Conclusion

It can be concluded that photobiomodulation with infrared LED irradiation reduces hospitalization time and eliminates the need for ICU admission or mechanical ventilation. Photobiomodulation therapy can be used as a complement to conventional treatment of COVID-19, promoting the improvement of cardiopulmonary functions and minimization of respiratory symptoms, suggesting that photobiomodulation therapy could reduce or non-use of corticosteroids.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval

This study was performed in line with the principles of the Declaration of Helsinki. It was approved by the Research Ethics Committee of the Anhembi Morumbi University (CAAE; 36,988,320.5.0000.5492) and registered with the Brazilian Registry of Clinical Trials (ReBEC) under the code U1111–1261-1981 (16/11/2020).

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Contribution to the Work by each Author

Pâmela Camila Pereira (doctoral student): conception and design of the study, data acquisition, data analysis, drafting the manuscript.

Carlos José de Lima: conception and design of the study.

Adriana Barrinha Fernandes: design of the study, data analysis, interpretation of data.

Renato Amaro Zângaro: revising the manuscript critically for important intellectual content.

Antonio Balbin Villaverde (student's advisor): conception and design of the study, statistical analysis, drafting the manuscript, final approval of the version, corresponding author.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

PCM thanks the Coordination for the improvement of Higher Education Personnel (CAPES), the PhD scholarship.

References

[1] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, Lancet 395 (2020) 497–506, https://doi.org/10.1016/S0140-6736(20)30183-5.
[2] T. Liang, Handbook of COVID-19 prevention and treatment, Zhejiang Univ. School Med. (2020), https://doi.org/10.1089/photob.2021.0050.
[3] D.B. Pascoal, A.C.S. Carvalho, L.E.L.F.S. Mata, et al., Acute respiratory syndrome: an exacerbated immune response to COVID19, Braz. J. Hea. Rev. 3 (2020) 2978–2994, https://doi.org/10.34119/bjhrv3n2-138.
[4] S.A. Lauer, K.H. Grantz, Q. Bi, et al., The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application, Ann. Intern. Med. 172 (9) (2020) 577–582, https://doi.org/10.7326/M20-0504.
[5] C. Leung, Clinical features of deaths in the novel coronavirus epidemic in China, Rev. Med. Virol. 30 (3) (2020), e2103, https://doi.org/10.1002/rmv.2103.
[6] S. Murthy, C.D. Gomersall, R.A. Fowler, Care for critically ill patients with COVID-19, JAMA 323 (15) (2020) 1499–1500, https://doi.org/10.1001/jama.2020.3633.
[7] D. Chen, W. Xu, Z. Lei, et al., Recurrence of positive SARS-CoV-2 RNA in COVID-19: a case report, Inter. J. Infec. Dis. 93 (2020) 297–299, https://doi.org/10.1016/j.ijid.2020.03.003.
[8] E. Terpos, I. Ntanasis-Stathopoulos, I. Elalamy, et al., Hematological findings and complications of COVID-19, Am. J. Hematol. 95 (7) (2020) 834–847, https://doi.org/10.1002/ajh.25829.
[9] Z. Sahraei, M. Shabani, S. Shokouhi, et al., Aminoquinolines against coronavirus disease 2019 (COVID19): chloroquine or hydroxychloroquine, Int. J. Antimicrob. Agents 55 (2020), 105945, https://doi.org/10.1016/j.ijantimicag.2020.105945.
[10] S.A. Baron, C. Devaux, P. Colson, et al., Teicoplanin: an alternative drug for the treatment of coronavirus COVID-19? Int. J. Antimicrob. Agents 55 (2020), 105944 https://doi.org/10.1016/j.ijantimicag.2020.105944.
[11] P. Gautret, J.C. Lagier, P. Parola, et al., Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int. J. Antimicrob. Agents 56 (1) (2020), 105949, https://doi.org/10.1016/j.ijantimicag.2020.105949.
[12] World Health Organization, Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected, Interim Guid. (2020) 1–10. Available at, https://apps.who.int/iris/handle/10665/330893.
[13] B.M. Kajagar, A.S. Godhi, A. Pandit, et al., Efficacy of low level laser therapy on wound healing in patients with chronic diabetic foot ulcers-a randomised control

trial, Indian J. Surg. 74 (5) (2012) 359–363, https://doi.org/10.1007/s12262-011-0393-4.
[14] I.I. Kerppers, C.J. de Lima, A.B. Fernandes, et al., Effect of light-emitting diode (λ 627 nm and λ945 nm) treatment on first intention healing: immunohistochemical analysis, Lasers Med. Sci. 30 (1) (2015) 397–401, https://doi.org/10.1007/s10103-014-1668-3.
[15] F.M. de Lima, L.M. Moreira, A.B. Villaverde, et al., Low-level laser therapy (LLLT) acts as cAMP-elevating agent in acute respiratory distress syndrome, Lasers Med. Sci. 26 (3) (2011) 389–400, https://doi.org/10.1007/s10103-010-0874-x.
[16] M.R. Hamblin, Mechanisms and applications of the anti-inflammatory effects of photobiomodulation, AIMS Biophys. 4 (3) (2017) 337–361, https://doi.org/10.1007/s12262-011-0393-4.
[17] P.C. Pereira, C.J. de Lima, A.B. Fernandes, et al., Systemic effects of photobiomodulation on blood components in the treatment of community-acquired pneumonia, Photobiomodul. Photomed. Laser Surg. 40 (1) (2021) 51–58, https://doi.org/10.1089/photob.2021.0050.
[18] K.C. Smith, Laser (and LED) therapy is phototherapy, Photomed. Laser Surg. 23 (2005) 78–80, https://doi.org/10.1089/pho.2005.23.78.
[19] A. Plessas, Nonsurgical periodontal treatment: review of the evidence, Oral Health Dent Manag. 13 (2014) 71–80.
[20] A.A. de Brito, E.C. da Silveira, N.C. Rigonato-Oliveira, et al., Low-level laser therapy attenuates lung inflammation and airway remodeling in a murine model of idiopathic pulmonary fibrosis: relevance to cytokines secretion from lung structural cells, J. Photochem. Photobiol. B 203 (2020), 111731, https://doi.org/10.1016/j.jphotobiol.2019.111731.
[21] F. Anastasio, S. Barbuto, E. Scarnecchia, et al., Medium-term impact of COVID-19 on pulmonary function, functional capacity and quality of life, Eur. Respir. J. 58 (2021) 2004015, https://doi.org/10.1183/13993003.04015-2020.
[22] De B.T.L. Matos, D.V. Buchaim, K.T. Pomini, et al., Photobiomodulation therapy as a possible new approach in COVID-19: a systematic review, Life (Basel) 11 (6) (2021) 580, https://doi.org/10.3390/life11060580.
[23] A. Domínguez, S.A. Velásquez, M.A. David, Can transdermal photobiomodulation help us at the time of COVID-19? Photobiomodul. Photomed. Laser Surg. 38 (5) (2020) 258–259, https://doi.org/10.1089/photob.2020.4870.
[24] A.B. Fernandes, C.J. de Lima, A.B. Villaverde, et al., Photobiomodulation: shining light on COVID-19, Photobiomodul. Photomed. Laser Surg. 38 (7) (2020) 395–397, https://doi.org/10.1089/photob.2020.4882.
[25] G. Litscher, L.M. Ailioaie, Comments on new integrative photomedicine equipment for photobiomodulation and COVID-19, Photonics 8 (2021) 303, https://doi.org/10.3390/photonics8080303.
[26] M. Nejatifarda, S. Asefib, R. Jamalic, et al., Probable positive effects of the photobiomodulation as an adjunctive treatment in COVID-19: a systematic review, Cytokine 137 (2021), 155312, https://doi.org/10.1016/j.cyto.2020.155312.
[27] C.P. Sabino, A.R. Ball, M.S. Baptista, et al., Light-based technologies for management of COVID-19 pandemic crisis, J. Photochem. Photobiol. B 212 (2020), 111999, https://doi.org/10.1016/j.jphotobiol.2020.111999.
[28] S.A. Sigman, S. Mokmeli, M. Monici, et al., A 57-year-old African American man with severe COVID-19 pneumonia who responded to supportive photobiomodulation therapy (PBMT): first use of PBMT in COVID-19, Am. J. Case Rep. 21 (2020), e926779, https://doi.org/10.12659/AJCR.926779.
[29] S.A. Sigman, S. Mokmeli, M.A. Vetrici, Adjunct low level laser therapy (LLLT) in a morbidly obese patient with severe COVID-19 pneumonia: a case report, Can. J. Respir. Ther. 56 (2020) 52–56, https://doi.org/10.29390/cjrt-2020-022.
[30] M.A. Vetrici, S. Mokmeli, A.R. Bohm, et al., Evaluation of adjunctive Photobiomodulation (PBMT) for COVID-19 pneumonia via clinical status and pulmonary severity indices in a preliminary trial, J. Inflamm. Res. 14 (2021) 965–979, https://doi.org/10.2147/JIR.S301625.
[31] T. de Marchi, F. Frâncio, J.V. Ferlito, et al., Effects of photobiomodulation therapy combined with static magnetic field (PBMT-sMF) in patients with severe COVID-19 requiring intubation: a pragmatic randomized placebo-controlled trial, J. Inflamm. Res. 14 (2021) 3569–3585, https://doi.org/10.2147/JIR.S318758.
[32] M.J. Fine, T.E. Auble, D.M. Yealy, et al., A prediction rule to identify low-risk patients with community acquired pneumonia, N. Engl. J. Med. 336 (1997) 243–250, https://doi.org/10.1371/journal.pone.0210102.
[33] C.P.C. de Jager, P.C. Wever, E.F.A. Gemen, et al., The neutrophil-lymphocyte count ratio in patients with community-acquired pneumonia, PLoS One 7 (2012), e46561, https://doi.org/10.1371/journal.pone.0046561.
[34] A. Hassan, Z. Ali, H. Iftikhar, et al., An emerging marker predicting the severity of Covid-19: neutrophil-lymphocyte count ratio, P. J. M. H. S. 15 (2021) 3398–3399, https://doi.org/10.53350/pjmhs2115113398.
[35] S. Jimeno, P.S. Ventura, J.M. Castellano, et al., Prognostic implications of neutrophil-lymphocyte ratio in COVID-19 Eur, J. Clin. Invest. 151 (1) (2021), e13404, https://doi.org/10.1111/eci.13404.
[36] S. Sawilowsky, New effect size rules of thumb, JMASM 82 (2009) 597–599, https://doi.org/10.22237/jmasm/1257035100.
[37] J. Cohen, Statistical Power Analysis for the Behavioral Sciences, 2nd ed., Lawrence Erlbaum Associates, New Jersey, USA, 1988.
[38] G. da Cunha Moraes, L.B. Vitoretti, A.A. de Brito, et al., Low-level laser therapy reduces lung inflammation in an experimental model of chronic obstructive pulmonary disease involving P2X7 receptor, Oxidative Med. Cell. Longev. 2018 (2018) 6798238, https://doi.org/10.1155/2018/6798238.
[39] M. Da-Palma-Cruz, R.F. Da Silva, D. Monteiro, et al., Photobiomodulation modulates the resolution of inflammation during acute lung injury induced by sepsis, Lasers Med. Sci. 34 (1) (2019) 191–199, https://doi.org/10.1007/s10103-018-2688-1.
[40] J. Phua, L. Weng, L. Ling, et al., Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations [published correction appears in Lancet Respir Med. 2020 May;8(5): e42], Lancet Respir. Med. 8 (5) (2020) 506–517, https://doi.org/10.1016/S2213-2600(20)30161-2.
[41] S.S. Tomazoni, D.S. Johnson, E.C.P. Leal-Junior, Multi-wavelength photobiomodulation therapy combined with static magnetic field on long-term pulmonary complication after COVID-19: a case report, Life (Basel) 11 (11) (2021) 1124, https://doi.org/10.3390/life11111124.
[42] A.P. Nair, A. Soliman, M.A. Al Masalamani, et al., Clinical outcome of eosinophilia in patients with COVID-19: a controlled study, Acta Biomed 91 (4) (2020), e2020165, https://doi.org/10.23750/abm.v91i4.10564.
[43] A.U. Anka, M.I. Tahir, S.D. Abubakar, et al., Coronavirus disease 2019 (COVID-19): an overview of the immunopathology, serological diagnosis and management, Scand. J. Immunol. 93 (4) (2021), e12998, https://doi.org/10.1111/sji.12998.
[44] B.M. Tomazini, I.S. Maia, A.B. Cavalcanti, Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19 – the CoDEX randomized clinical trial, Jama 324 (13) (2020) 1307–1316, https://doi.org/10.1001/jama.2020.17021.
[45] H.C. Prescott, T.W. Rice, Corticosteroids in COVID-19 ARDS – evidence and hope during the pandemic, Jama 324 (13) (2020) 1292–1295, https://doi.org/10.1001/jama.2020.16747.
[46] The WHO rapid evidence appraisal for COVID-19 therapies (React) working group, Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19, JAMA 324 (34) (2020) 1298–1307, https://doi.org/10.1001/jama.2020.17023.